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Myasthenia Gravis

These discussions are selected from the weekly staff conferences in the Department of Medicine, University of California, San Francisco. Taken from transcriptions, they are prepared by Drs. David W. Martin, Jr., Associate Professor of Medicine, and James L. Naughton, Assistant Professor of Medicine, under the direction of Dr. Lloyd H. Smith, Jr., Professor of Medicine and Chairman of the Department of Medicine. Requests for reprints should be sent to the Department of Medicine, University of California, San Francisco, CA 94143.

DR. SMITH:* *Recently, a patient in the intensive care unit with myasthenia gravis was treated with plasma exchange, the first time this treatment had been used for myasthenia in this hospital. Now seems to be an opportune time to have Dr. Robert Layzer review myasthenia gravis and describe some recent advances in our understanding of the pathogenesis of this neurological condition.*

DR. LAYZER:† Treatment of myasthenia has made steady progress through the years, but until recently knowledge about pathogenesis and treatment remained more or less separate, although they now seem to be converging rapidly.

I thought that the best way to review this subject would be to go back to 1960, which was a turning point for the modern understanding of myasthenia gravis, and to trace historically the development of ideas since then. First, it was known in 1960 that myasthenia was a disorder of neuromuscular transmission, partly relieved by anticholinesterase drugs, but it was not certain whether the defect represented a poor response to nerve stimulation—that is, an abnormality on the postsynaptic side of the neuromuscular junction—or a presynaptic defect in the release of acetylcholine from the nerve terminal. Second, it was known that about 10 percent of patients with myasthenia gravis had a thymoma, and that those who did not have a thymoma usually had hyperplasia of the thymic lymphoid tissue.[1] Third, it was generally agreed (though the statistical evidence was somewhat weak) that removing the thymus gland in patients who did not have a thymoma was an effective form of treatment for myasthenia gravis.

To expand a little, it should be recalled that before 1960 the postoperative course of myasthenic patients was often stormy because of the primitive state of facilities for assisting ventilation. After the introduction of positive pressure ventilation in the late 1950's, thymectomy assumed increasing importance in the management of myasthenia, especially for patients who had more than minor symptoms. Table 1 lists the results of thymectomy in a recent study done at the Mayo Clinic.[2] Like other retrospective surveys of thymectomy in myasthenia gravis, it is somewhat lacking. However, the authors used a computer to match 80 surgically treated patients with 80 patients who were not operated on, so that the two groups were comparable in every important respect, including severity and duration of disease. The average duration of follow-up was approximately 20 years. As one can see, the number of patients in remission was four to five times higher in the group of surgically treated patients, the number of improved patients was double, and the number dead was less than half.

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TABLE 1.—*Results of thymectomy in 80 patients, compared with 80 matched patients who were not surgically treated. All patients were older than 16 years and were treated at the Mayo Clinic before 1965. The average follow-up was between 20 and 23 years.**

Status	Surgical Group (80)	Medical Group (80)
Complete remission	27	6
Improved	26	13
Unchanged	3	7
Worse	1	5
Dead	21	47
Myasthenia gravis	11	34
Other causes	10	13
Lost to follow-up	2	2

*Reproduced from Buckingham et al with permission from Ann Surg.[2]

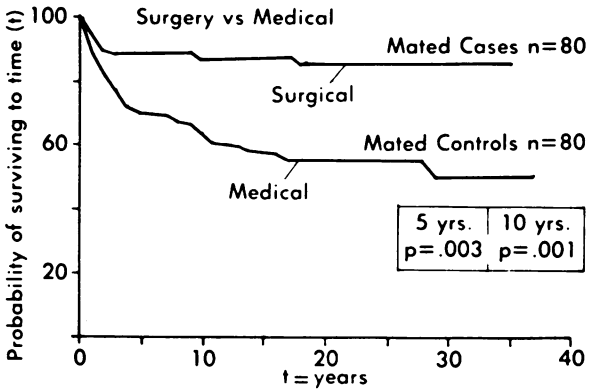

Figure 1.—Survival curve showing the effect of thymectomy in myasthenia gravis. (Reproduced from Buckingham JM et al, with permission from Ann Surg.[2])

Figure 1 shows these data in a survival curve; the increased death rate in cases without operations appeared to continue for about 15 years following the beginning of the study. Other studies, from many other institutions in several countries, have provided similar results. Now thymectomy is generally accepted as the treatment of choice in disabling myasthenia, and most authorities recommend that it be carried out early in the course of the disease, although there is little evidence to support the last point.[3]

Another important fact known in 1960 was that babies born to mothers with myasthenia gravis occasionally exhibited transient symptoms of myasthenia in the immediate postnatal period.[4] The weakness tended to be mild but sometimes required treatment with anticholinesterase drugs, and always disappeared in a few weeks. This transient neonatal myasthenia made many suspect that a circulating substance, capable of being transferred across the placenta, was present in the plasma of patients with myasthenia gravis. Through the years there were several attempts to show neuromuscular blocking activity in myasthenic serum by testing its effect on neuromuscular transmission in animals, either *in vitro* or *in vivo*. During one such study in the late 1950's, a medical student, Arthur Strauss, working at Columbia University with William Nastuk, discovered a cytopathic effect of myasthenic serum on muscle in frogs. This finding led to the discovery that circulating antibodies reacting with skeletal muscle were present in 30 percent of myasthenic patients, in 95 percent of patients with both myasthenia and thymoma, and in 24 percent of patients with thymoma who did not have myasthenia.[5,6] The antibodies reacted with the cross striations of muscle, not with the neuromuscular junction, which could not easily explain the physiological disorder. They also reacted with the epithelial cells of the thymus, a type of cell that was later discovered to be a primitive muscle cell, mysteriously present in the thymus gland of humans and lower animals. In 1960 Nastuk and Strauss published their hypothesis, based on the findings of these immunological studies, that myasthenia gravis was an autoimmune disease.[5]

Coincidentally, in the same year, J. A. Simpson, a Scottish neurologist, published in the *Scottish Medical Journal* a lecture in which he also proposed that myasthenia gravis was an autoimmune disease.[7] Arguing from clinical observations, he supposed that a circulating antibody was present which reacted with the acetylcholine receptor on the postsynaptic surface of the neuromuscular junction. This clairvoyant prediction was prompted by an apparent association that Simpson had detected between myasthenia gravis and certain immunological disorders, such as rheumatoid arthritis and pernicious anemia.

After the autoimmune theory was promulgated in 1960, some years went by before new supporting evidence was introduced. The next major advances, in fact, did not relate directly to the immunological question but to questions involving physiology and pathology. From 1950 to 1960 the details of neuromuscular transmission were clarified. A diagram of the neuromuscular junction is shown in Figure 2. The postsynaptic region of the muscle is arranged in deep folds forming troughs, at the mouths of which lie the acetycholine receptors. The nerve terminal contains many synaptic vesicles, each containing

several thousand molecules of acetylcholine. When a nerve impulse invades the nerve terminal, the contents of 50 to 100 synaptic vesicles are released simultaneously, producing a large depolarization of the postsynaptic membrane. At rest, however, individual packets of acetylcholine are also released spontaneously, at the rate of about one per second. These spontaneously released packets of acetylcholine produce small depolarizations of 0.5 to 1.0 mV in the postsynaptic membrane potential as recorded by a microelectrode in that region. The term *miniature end-plate potentials* is applied to such depolarizations. In 1964, Elmquist and his colleagues,[8] studying intercostal muscle biopsy specimens, showed that in myasthenia gravis the amplitude of the miniature end-plate potentials averaged a fifth of the normal amplitude (Figure 3). This striking abnormality could have resulted from a poor response of the postsynaptic membrane to acetylcholine or from a reduced release of acetylcholine from the nerve terminal; it was not immediately apparent which explanation was correct.

Seven years later, in 1971, Engel and Santa[9] helped to answer this question with their histometric studies of the electron-microscopic appearance of the neuromuscular junction, Figure 4. In many myasthenic end-plates the postsynaptic structure was quite abnormal, with considerable loss of membrane surface area and great simplification of the synaptic folds. In places, debris from the disintegration of the postsynaptic membrane was seen lying within the synaptic clefts.

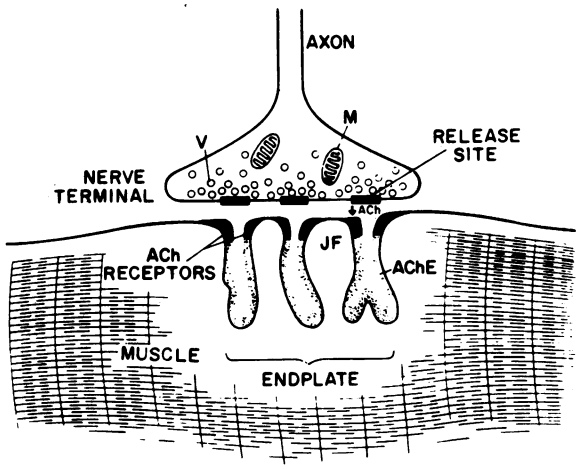

Figure 2.—Diagram of neuromuscular junction. Vesicles (V) release their acetylcholine (ACh) contents at specialized release sites. After crossing the narrow synaptic space **(path indicated by arrow)** ACh reaches the ACh receptors, which are situated most densely at the peaks of the junctional folds (JF). Acetylcholinesterase (AChE) in the clefts rapidly hydrolyzes the ACh. M denotes mitochondria. (Reproduced from Drachman DB, with permission from N Engl J Med.[13])

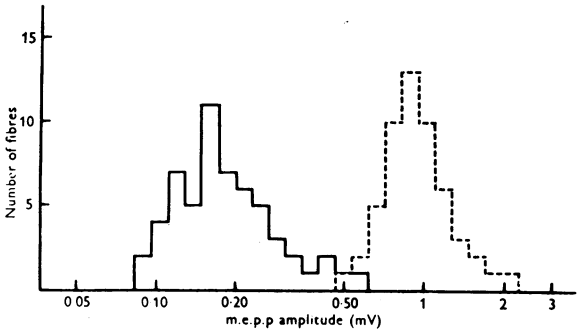

Figure 3.—Distribution of mean miniature end-plate potential (m.e.p.p.) amplitudes recorded from 57 myesthenic **(solid line)** and 54 normal **(broken line)** intercostal muscle fibers. Note logarithmic scale of abscissa. (Reproduced from Elmqvist D et al, with permission from J Physiol.[8])

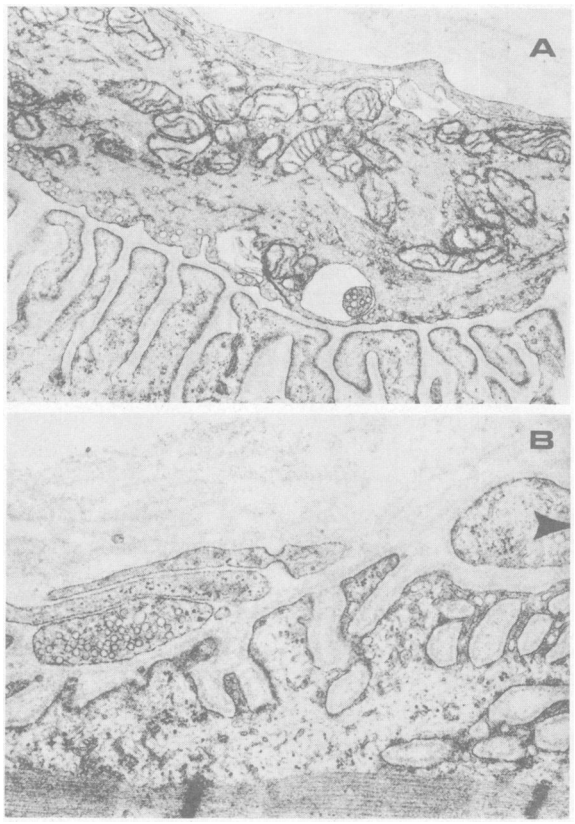

Figure 4.—Ultrastructure of neuromuscular junction in myasthenia gravis. **A,** a normal end-plate with numerous deep synaptic clefts formed by folding of the postsynaptic membrane. **B,** a myasthenic end-plate with shallow, sparse synaptic clefts. (Reproduced from Engel AG et al, with permission from Ann NY Acad Sci.[9])

The overall impression was of a degenerative process principally affecting the postsynaptic portion of the end-plate.

Meanwhile, a new phase in treatment was under way, perhaps inspired by the autoimmune hypothesis. In the late 1960's it was found that a short course of adrenocorticotrophic hormone (ACTH) in high doses caused a transient increase in myasthenic weakness, after which, for a period lasting a few weeks or months, patients became stronger than they were before treatment.[10] In 1972 the use of continuous high-dose prednisone treatment was introduced,[11] quickly replacing ACTH therapy. In most patients there was dramatic improvement, although during the early phase of treatment regression was often seen. It has been suggested that prednisone and other corticosteroids have a direct neuromuscular-blocking effect, but this has not been well established. After the condition of a patient improves, the harmful effect of prednisone is no longer prominent.

Long-term treatment with prednisone is now a major factor in the management of severe myasthenia gravis.[12] My view is that corticosteroid treatment should be reserved for patients who have disabling or life-threatening symptoms, usually after thymectomy has been done. It is unfortunate that in many patients there is no improvement for several years after thymectomy; therefore, therapy with prednisone often provides effective relief in those patients with severe myasthenia gravis in whom thymectomy has not been effective. In most patients, I initiate treatment with 40 to 60 mg of prednisone per day in divided doses, changing to an alternate-day schedule after improvement occurs, usually within three months. Slow tapering of the dosage is usually possible. Many patients regain normal muscular function during treatment, but there is no evidence that administration of prednisone shortens the duration of the disease. In other words, there is no reason to think that prednisone has a curative rather than a suppressive effect.

In 1973 myasthenia research advanced in a new direction, largely because of a snake venom called bungarotoxin.[13] This paralyzing protein binds specifically and irreversibly to the nicotinic acetylcholine receptor of many different organisms, from humans to electric eels. Recognizing this fact, investigators in several laboratories began to use bungarotoxin and related toxins for two purposes: (1) using affinity chromatography to purify the acetylcholine receptor, which is plentiful in tissues of electric eels and electric fish and (2) using bungarotoxin labeled with radioactive iodine (^{131}I) to study the number and turnover of acetylcholine receptors in muscle under a variety of experimental conditions. Fambrough and associates[14] estimated the number of acetylcholine receptors in myasthenic muscle by counting the radioactivity of bungarotoxin ^{131}I bound after incubation with an intercostal muscle biopsy specimen. In myasthenic end-plates, bound bungarotoxin radioactivity (measured directly or by autoradiography) was reduced to between 10 percent and 20 percent of normal. This was the first direct evidence that the defect of neuromuscular transmission in myasthenia gravis is postsynaptic, settling a controversy that had continued for several decades.

Meanwhile, at the Salk Institute, Patrick and Lindstrom[15] were purifying the acetylcholine receptor from electric eels. Intending to prepare antibodies against the purified receptor, they injected the receptor, emulsified in Freund's adjuvant, into rabbits. Within three weeks after the second injection several rabbits became paralyzed. The paralysis was reversed through the administration of anticholinesterase drugs, and repetitive nerve stimulation showed a decrementing response similar to that seen in myasthenia gravis. Patrick and Lindstrom realized that they had inadvertently created an experimental autoimmune model of myasthenia gravis. The new disease model was quickly adopted by other investigators, and the pathogenesis of the experimental disease was soon clarified.[16]

Following immunization with the acetylcholine receptor, there was a lymphocytic invasion of the end-plate region, leading to structural damage to the postsynaptic membrane concentrated at the mouths of the folds where the acetylcholine receptor is located. Next, antibody appeared in the blood, and a phase of continuing minor damage ensued, associated with persistent levels of antibody in the blood. It is interesting that the antibodies reacted primarily with the foreign species of acetylocholine receptor used for immunization, but later antibodies were more reactive with the animal's own acetylcholine receptors.

The next step was to search for antibodies acting against acetylcholine receptor in the serum of patients with myasthenia gravis. In the past all such attempts had been unsuccessful, but in

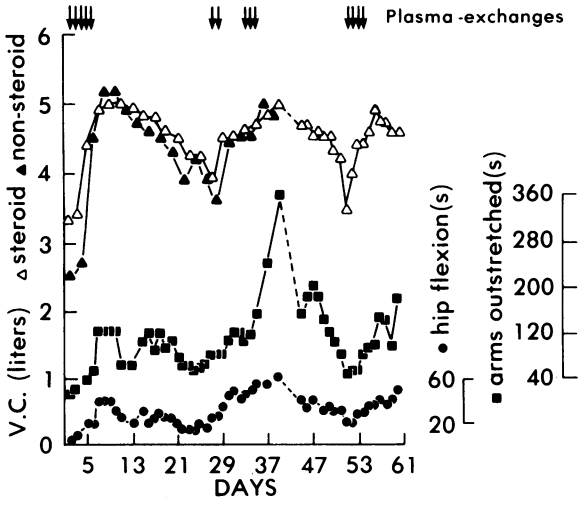

Figure 5.—Effect of plasma exchange therapy on muscle strength in a myasthenic patient receiving alternate-day prednisone treatment. Vital capacity (V.C.) and limb strength (recorded as number of seconds held up against gravity) improved transiently following each course of plasma exchanges. Three sets of plasma exchange (all of 2 litres) are marked, the second set being divided into two parts. Administration of pyridostigmine bromide was stopped on day 9; reduction in steroid dosage started on day 15. The dotted line indicates a time when measurements were not possible; after this time steroids were given every day. (Reproduced from Pinching AJ, with permission from Lancet.[21])

1974 and subsequently, new techniques, based on the use of bungarotoxin ^{131}I, enabled groups led by Appel,[17] Lindstrom[18] and others to detect antibodies to acetylcholine receptor in 80 percent to 90 percent of patients with myasthenia gravis. There is only a very rough correlation between the titer of these antibodies and the severity of the disease, but patients with purely ocular myasthenia lack antibody more often than those with generalized myasthenia gravis. Following thymectomy or steroid therapy, levels of antibody tend to fall as the patient improves.

It was tempting to assume that these antibodies played a role in causing myasthenia gravis in humans. However, might they not simply be an epiphenomenon—the result of damage to the motor end-plate rather than the cause? To answer this question, Drachman and colleagues,[19] in 1975, injected large amounts of a globulin fraction of myasthenic serum into mice. They found that the amplitude of miniature end-plate potentials was reduced more than 50 percent, and many of the mice appeared weak—an effect not found with normal human serum. Meanwhile, in 1973 Bergstrom and associates[20] reported that continuous drainage of lymph from the thoracic duct of myasthenic patients resulted in temporary improvement, which could be reversed by infusion of the cell-free lymph or of an immunoglobulin fraction of the lymph. This observation led to the most dramatic recent development in the treatment of myasthenia. In 1976 Pinching and co-workers[21] reported temporary but striking improvement of weakness in three myasthenic patients treated with plasma exchange (Figure 5). Later the authors documented a fall in antibody titers during the clinical improvement, and a rise in titers as symptoms returned.[22] The beneficial effect could be prolonged in many cases by immunosuppressive therapy at the time of plasma exchange. Similar results have been reported by Dau and associates in San Francisco.[23] Plasma exchange (plasmapheresis) is now being used rather widely, but primarily as a temporary treatment for seriously ill patients while awaiting the benefits of the slowly acting immunosuppressive drugs.

These results leave little doubt that serum antibodies play a continuing pathogenic role in most patients with myasthenia. How do the antibodies exert their harmful effects? Although the structural damage at the motor end-plate, which is a pathological feature of myasthenia, could be antibody mediated, it seems unlikely that this damage could be repaired quickly enough to account for the early clinical response to plasma exchange. The antibody could have a simple receptor-blocking action, like curare, but Engel and co-workers[24] cast some doubt on this explanation. In 1977 they showed that immune complexes (IgG and the C3 component of complement) are deposited on the postsynaptic membrane of myasthenic motor end-plates. The length of postsynaptic membrane containing either IgG or C3 was directly proportional to the size of miniature end-plate potentials, as determined by electrophysiological measurements (Figure 6). Therefore, the more antibody found at the end-plate, the better was neuromuscular transmission. Presumably the antibodies, like bungarotoxin ^{131}I in earlier studies, provided an index of the number of available acetylcholine receptors without blocking transmission. Finally, it has been shown that myasthenic immunoglobulin accelerates the degradation of acetylcholine receptors,[25] and this process has a time course that could account for the rapid clinical response to the removal of antibodies.

We have seen how recent developments have led to a more or less rational approach to the

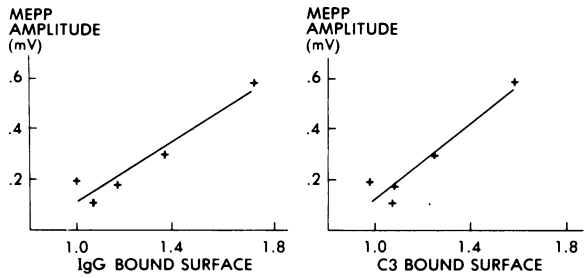

Figure 6.—Relationship between mean miniature end-plate potential (MEPP) amplitude and amount of IgG and C3 bound to postsynaptic membrane. Data are from intercostal muscle biopsies of five myasthenic patients. MEPP amplitude is directly proportional to the surface area occupied by immune complexes, suggest-that these complexes do not directly inhibit neuromuscular transmission. (Reproduced from Engel AG, with permission from Proc Mayo Clinic.[24])

treatment of myasthenia gravis as an immunological disorder. I think two major questions about pathogenesis remain for future research: What is the role of the thymus gland and what initiates the autoimmune reaction? A more specific way of reversing the immunological attack on the motor end-plate without interfering with normal defense mechanisms is needed for treatment of myasthenia. Judging from the rate of recent progress, we may not have to wait long for solutions to these problems.

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